GAS-LIQUID CHROMATOGRAPHY OF METHYL ETHERS OF HIGHER FATTY ACIDS OF SENDAI VIRUS LIPID COMPONENT

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GAS-LIQUID CHROMATOGRAPHY OF METHYL ETHERS OF HIGHER FATTY ACIDS OF SENDAI VIRUS LIPID COMPONENT

[Following is the translation of an article by G. A. Smirnova and A. Yu. Virkus, Institute of Virology imeni D. I. Ivanovskogo, AMN, USSR, Moscow, published in the Russian-language periodical Voprosy Virusologiya (Problems of Virology), 1964, No 4, pages 417-421. Translation performed by Sp/7 Charles T. Ostertag Jr.]

A significant content of lipids has been detected in the composition of many viruses of animals. Thus, based on the data of several authors [5, 6], their content in highly purified preparations of influenza virus reaches 47.9%. In the total lipid fraction it was possible to identify 12 compounds, among which were polar lipids -- free higher fatty acids, neutral fat, and steroids. In regards to the parainfluenza Sendai virus, data concerning its chemical composition has not been detected in the literature available to us.

The aim of the present investigation was a study of the fatty acid composition of the petroleum ether fraction of the lipid component of the Sendai virus.

Materials and Methods

In the work the LM-1 strain of the Sendai parainfluenza virus was used. It was cultivated on 10-11 day chick embryos at a temperature of 35° for 72 hours following their infection in the allantoic cavity with a 1,000 EID_{50} dose of the virus. The virus containing allantoic fluid with a titer of hemagglutinins of 1:5120 was collected from the refrigerated embryos.

The virus was subjected to concentration and purification, for which we used: 1) adsorbtion on formalin treated chick erythrocytes with subsequent elution with a physiological solution of sodium chloride; 2) differential centrifugation at low (1600 g) and high (27,000 g) velocities; 3) filtration through G-75 SEPHADEX gel [2].

The purified preparations of the virus, which possessed a high hemagglutinating activity (the titer of agglutinins was higher than

1:160,000,000) and preserved the infectious properties, were lyophilized. In the dry preparations the content of total nitrogen and protein was determined [8], and also the degree of purification of the preparations with the help of paper electrophoresis [7]. Then 1 g of dry preparation of the virus was subjected to successive exhaustive extraction with organic solvents (petroleum ether, sulfuric ether, chloroform and alcohol ether) according to the generally accepted methods [4]. In each fraction a determination was made of the total content of extracted substances in relation to the weight of the preparation used.

In the petroleum ether fraction the quantitative and qualitative content of higher fatty acids (both free as well as bound) were determined by the method of gas chromatography[13, 14]. For this we used the UKh-1 (*) chromatograph, put out by the Tallin Plant of regulating-metering devices. The chromatograph has a detector, which essentially is a two-channel catharometer, equipped with tungsten filaments, forming the two arms of the Wheatstone bridge.

[Translator's note: (*) Probable meaning of the abbreviation UKh -- Uitston Khromatograf, Wheatstone bridge chromatograph.]

With the help of this device it is possible to separate substances with boiling points of -269° up to +300°, and also to isolate individual fractions for their spectral investigation. The working temperature of the device is 20--210°. Hydrogen or helium are used as gas-carriers. In our tests helium was used. The velocity of the gas flow was from 20 to 200 ml/min. The volume of the specimens of gasses being tested comprised 1--10 ml, with liquid -- 1--50 mkl. The duration of analysis for various substances was from 10 sec. to three hours.

Results

When working on the UKh-1 chromatograph the necessity arose for selecting the carrier and the liquid phase. In the capacity of the solid phase, most often Celite 545 and Chromosorb are used [9, 11]. However, these materials are little accessible due to their shortage. As the solid phase we used diatomite, INZ-600, and sponge glass [1], and as the liquid phase -- silicone oil and polyethylene-glycol succinate [13, 14].

The sponge glass was prepared according to the following method [1]. Ordinary laboratory glass No. 23 was ground in a bead grinder, and the resulting powder was sintered for 24 hours at a temperature of 450°. The clumps of sponge glass were ground, sifted, and then the

fraction with a particle diameter of 1--0.25 mm was removed. This was treated with concentrated hydrochloric acid, washed with water and dried. To it, 1% trimethylchlorosilane dissolved in acetone was added. The solvent was withdrawn at room temperature, after which the processed material was heated for two hours at 200°.

The synthesis of polyethylene-glycol succinate was conducted according to the method of Karoly et al. [3, 12] in our modification. Into a three-necked flask, equipped with a turbine agitator, air cooler and thermometer, we introduced 60 g of succinic acid, 45 g of ethylene glycol, and 100 mg of anhydrous zinc chloride as a catalyst. The mixture was heated for three hours at a temperature of 200° up until discontinuance of water separation. Then the air cooler was replaced by a downflow and gradually increased the temperature in the course of an hour up to 250°, and the pressure was lowered to 3 mm mercury column. The mixture was maintained under these conditions up until a constant viscosity was achieved. As a result a white solid mass was obtained with a melting point of 82--83°.

Throughout the entire reaction a stream of propanol-butanol was passed through the mixture for creating an inert medium.

A solution of silicone oil in acetone or polyethylene-glycol succinate in chloroform was added to the solid phase and it was processed by the same method as the sponge glass after the addition of trimethylchlorosilane. The filler prepared by such a method was purified from dust and large particles, and under the influence of vibration it was used to fill the copper columns with a length of 3200 mm and an internal diameter of 4 mm. The filled columns were bent into a spiral.

For testing the columns with various carriers we prepared a mixture from saturated and unsaturated higher fatty acids of the following composition: Myristic acid -- 19.2%, palmitic -- 39.7%, stearic -- 16.9%, oleic -- 24.2%. The latter was isolated from sunflower oil [7]. For lowering the boiling point of the higher fatty acids and decreasing polarity they were transferred into methyl ethers by diazomethane [14]16].

The first tests were carried out using diatomite and silicon oil (1% based on the weight of diatomite). These materials turned out to be unsuitable in view of the illegibly formed peaks on the chromatogram. An analogous picture was observed with INZ-600 and silicon oil. An unsatisfactory result was obtained when using sponge glass and silicon oil. The asymmetrical position of the peaks and the incomplete separation of individual components of the mixture pointed to the unsuitability of silicon oil as the liquid phase. In connection with this we used

polyethylene-glycol succinate (5% of the weight of the solid carrier) as the liquid phase. The chromatogram, obtained with the separation on this filter of the mixture of methyl ethers of fatty acids, is shown on figure 1.

As is apparent in figure 1, sponge glass with polyethylene-glycol succinate guarantees the fine separation of individual components of the mixture. For the qualitative identification of individual components of the mixture we passed each of the ethers separately through the column, and subsequently a standard mixture of the methyl ethers of the stated fatty acids. On the chromatograms of the mixtures the plateaus of the peaks of each ether of the mixture were measured with a planimeter, and based on the data obtained a graph was constructed for the corresponding fatty acid (fig. 2)

During comparative investigations of the mixtures the error amounted to $\pm$ 1%. By the described method we determined the fatty acid content of the petroleum ether fraction of the Sendai virus lipid component.

Saponification of the tests was conducted according to the generally accepted method with a 2N alcohol solution of KOH. The free fatty acids were isolated by acidification with a 10% solution of hydrochloric acid and extraction with ethyl ether. The ether extract was washed three times with distilled water and dried with anhydrous sodium sulfate. The dry ether extract was treated with a solution of diazomethane and with a microsyringe it was introduced into the column for chromatographic investigation.

Figure 3 presents the chromatogram of the methyl ethers of the higher fatty acids of the petroleum ether fraction of the Sendai virus lipid component. By using a calibration curve, we determined the overall content (in percentages) of fatty acids of this virus.

Palmitic acid	20.5
Stearic acid	30.0
Oleic acid	35.0
Linoleic acid	14.5

Conclusions

1. By using the method of gas-liquid chromatography with sponge glass (solid phase) and polyethylene-glycol succinate (liquid phase) as the filler, a quantitative and qualitative determination was performed of the fatty acids found in the composition of the Sendai virus.

2. The proposed method may be used for the quantitative and qualitative analysis of fatty acids found in the composition of the lipid component of animal viruses.

Literature

1. Kestner, A. I., Zh. fiz. khimii (Journal of Physical Chemistry), 1963, No 3, page 707.

2. Smirnova, G. A., Levyant, M. I., Vopr. virusol. (Problems of Virology), 1963, No 4, page 514.

3. Synthesis of Organic Preparations, Moscow, 1952, Coll. 3, page 277.

4. Pekh, K., In the book: Biochemical Methods of Plant Analysis, Moscow, 1960, page 23.

5. Armbruster, O., Beiss, H., Z. Naturforsch., 1958, Bd 1313, s 75.

6. Frommhagen, L. H., Greeman, N. K., Knight, C. A., Virology, 1958, V 5, page 173.

7. Keil, B., Sormova, Z., Laboratorni technika Biochemie, Prague, 1959, pages 137, 434.

8. Lowry, O. H., Rosebrough, N. J., et al., J. Biol. Chem., 1951, V 193, page 265.

9. Stuve, W., Fette, Seifen, Anstrichmittel., 1961, Bd 63, s 325.

10. Janus, A. F., J. Chromatogr., 1959, V 2, page 552.

11. Magidman, P., Herb, S. F., Barford, R. A., et al., J. Am. Oil. Chemists. Soc., 1962, V 39, page 137.

12. Koroly, J. E., Beavers, E. M., Indust. and Engng. Chem., 1953, V 45, page 1060.

13. Smith, L. M., J. Dairy Sci., 1961, V 44, page 607.

14. Craig, B. M., Murty, N. L., J. Am. Oil. Chemists. Soc., 1959, N 11, page 549.

15. Endars, J. G., Bholirno, W. R., Kuminera, F. A., J. Am. Oil. Chemists. Soc., 1962, V 39, page 118.

16. Vorbeck, M. L., Mattick, L. R., Lee, F. A., et al, Analyt. Chem., 1961, V 33, page 1512.

Figure 1. Chromatogram of an artificial mixture of fatty acids; polyethyleneglycol succinate -- 5%, sponge glass. Helium 40 ml/min. Temperature 200°.

0 - admission; 1 - ethyl ether; 2 - myristic acid; 3 - palmitic acid;
4 - stearic acid; 5 - oleic acid.

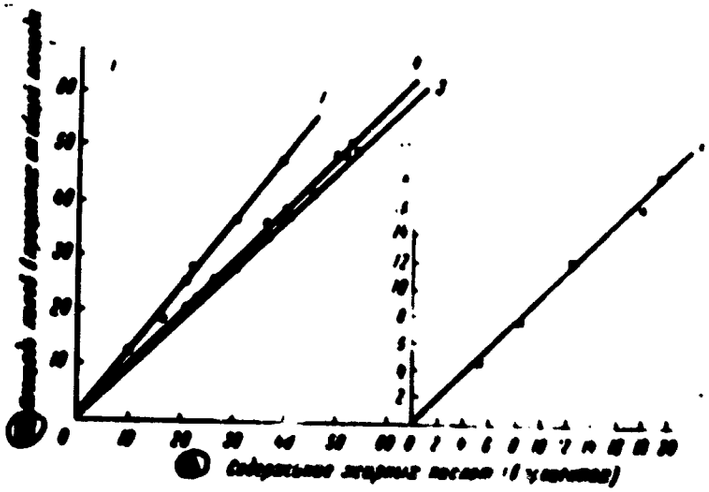

Figure 2. Calibration curves. A - content of fatty acids (in percentages); B - peak plateaus in percentages from total plateau. 1 - palmitic acid; 2 - stearic acid; 3 - oleic acid; 4 - linoleic acid.

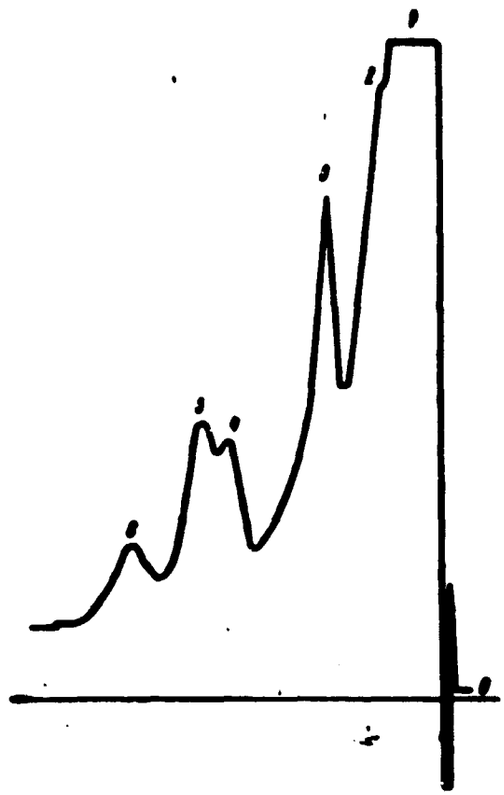

Figure 3. Chromatogram of the fatty acids from the petroleum ether fraction of the Sendai virus lipid component. 0 - admission; 1 - ethyl ether; 2 - myristic acid; 3 - palmitic acid; 4 - stearic acid; 5 - oleic acid; 6 - linoleic acid. Helium 45 ml/min. Temperature 200°.